

Medfly (Diptera:Tephritidae) Genetic Sexing: Large-Scale Field Comparison of Males-Only and Bisexual Sterile Fly Releases in Guatemala

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ABSTRACT The effect of releases of bisexual (males and female) and unisexual (male only) sterilized medflies was compared in three large field evaluations over a 3-yr period (1995–1997) in southwestern Guatemala. The two strains tested were a genetic sexing strain, Vienna-4/Tol-94, carrying the temperature sensitive *tsl* gene to eliminate females in the egg stage, and the standard bisexual Petapa strain. Flies were mass-reared, sterilized by irradiation as pupae, shipped to a field center, and released by air as young adults over 2 km by 2 km core areas in the centers of separate 6 km by 6 km test plots. Strain performance was monitored weekly by trapping sterile and wild male adults in core and buffer areas and by collecting eggs from coffee berries to determine induced sterility. Results indicated a several-fold advantage for the males-only strain as measured by the level of induced sterility, especially at the very high release ratios of 100:1 recorded in 1997. During that final test year, sterile-fly release rates were increased to provide high sterile:wild (S:W) fly ratios in the field, and egg sterility reached levels in excess of 70% in plots where the male-only strain was used. However, in the plots where the bisexual strain was released, induced sterility only reached 12% despite S:W ratios above 1,000:1.

KEY WORDS field evaluation, induced sterility, males-only releases, genetic sexing, *Ceratitis capitata*

THE STERILE INSECT TECHNIQUE has been used effectively in several areas around the world for suppression or control of resident populations of the Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wied.) (Steiner et al. 1962, Rhode et al. 1971, Fisher et al. 1985, Ortiz et al. 1987). Successes in eliminating incipient infestations of this species have also been reported (Cunningham et al. 1980, Wong et al. 1986).

During recent years, the development of genetic sexing strains (GSS) for this species has been achieved. These special strains allow the separation of the sexes during development based on a genetic mechanism such as, for example, pupal coloration, body size, or developmental rate. The aim of applying this type of technology is to increase the efficiency of the sterile insect technique when only males are released (Franz et al. 1994, 1996, Hendrichs et al. 1995). However, there have been only a small number of field tests demonstrating the potential of an all male release. In small scale field tests, McInnis et al. (1994) showed that releases of sterilized male medflies could induce

several times more sterility into wild medfly populations compared with when both males and females were released. Initial field cage tests also showed that males when released alone were able to mate with a higher proportion of wild females than when they were released together with females (Robinson et al. 1986). However, later field cage tests have been unable to confirm this observation. The initial GSS were not stable enough to be mass reared in large numbers (Rössler 1982 a, b, Hooper et al. 1987, Busch-Petersen 1989, Busch-Petersen and Kafu 1989). Improved GSSs are now available (Franz et al. 1994), and they are now being mass reared in large numbers at several facilities (Cáceres et al. 2003). The ability to mass rear this strain enables large-scale field studies to measure sterility induced into wild populations by comparing the relative effectiveness of bisexual versus unisexual (males-only) releases.

Prior field studies, using released sterile males and females of standard strains, have varied in the size of the test area: 25 km² (Fisher et al. 1985), 13 km² (Wong et al. 1986), 5 km² (Nitzan et al. 1993), 3.7 km² (Cirio et al. 1987), and 0.01–0.04 km² (McInnis et al. 1994). Release ratios (sterile:wild) have also varied from 50:1 (Wong et al. 1986) to overflooding rates of 200:1–1,000:1 (Fisher et al. 1985, McInnis et al. 1994). In addition, sampling data used to validate population suppression by the sterile insect technique have in-

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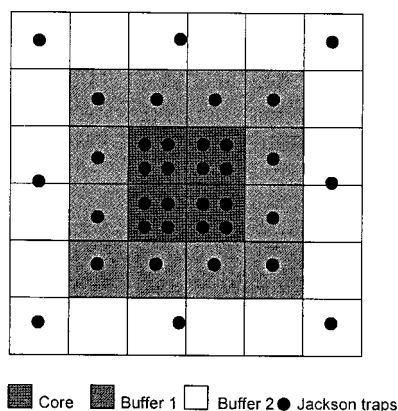


Fig. 1. Layout of each of the plots where either VIENNA 4/Tol-94 or standard strains were released (each square = 1 km²; Guatemala, 1995–1997). Total size of plot = 6 km × 6 km = 36 km².

cluded adult trap catches, fruit infestation, and percent egg hatch, with egg hatch being regarded as the most reliable index of success (Wong et al. 1986, McInnis et al. 1994). Other studies have used the same indices but given them a different order of priority (Cirio et al. 1987, Nitzan et al. 1993).

This study compared induced sterility in the field when (1) males and females of a bisexual strain (Petapa) were released and (2) predominantly males from the VIENNA-4/Tol-94 temperature-sensitive lethal GSS were released. This study provides the first data from a large-scale field trial designed to compare the relative merits of the two release scenarios. During 3 consecutive yr, induced egg sterility was measured in wild females after releases of >1 million sterile medflies/wk from the GSS VIENNA 4/Tol-94 and the Petapa bisexual strain. Flies were released in separate 4-km² field sites in Guatemala, and key parameters such as sterile:wild fly ratios and induced egg sterility were measured.

Materials and Methods

Testing Areas. The test area was located in Retalhuleu Department (Southwestern Guatemala), 176 km from Guatemala City. Facilities for quality control tests and aerial release procedures were close to the individual test sites. There was a large resident medfly population in the coffee (*Coffea arabica* L.) plantations.

Two experimental plots (6 km by 6 km) situated at about the same altitude (600–800 m above sea level) were selected for the releases. The plots were 6 km apart. Aerial releases of sterile medfly were carried out for several weeks over a core area of 4 km² (2 km by 2 km) in each of 3 consecutive yr (1995–1997; Fig. 1). During 1995, the plot in Palajunoy (latitude, 14°40'20"; longitude, 91°37'23") received flies from VIENNA 4/Tol-94, whereas the second plot, Asintal (latitude, 14°40'; longitude, 91°45'), received flies from the

Petapa strain. In 1996, the release locations were reversed. During 1997, the location for release of each strain remained the same as in 1996. Each plot had two buffer zones (B1–B2) surrounding the core area (C; Fig. 1). A third plot, in Mujulia (latitude, 14°44'12"; longitude, 91°47'28"), located 4.1 km from Asintal, served as a control area from where egg samples were obtained to measure control sterility rates in a natural population.

Trapping. Sixteen Jackson traps were placed inside the core area (Fig. 1) to determine (1) the distribution of released insects and (2) the ratio of sterile:wild male flies. Each of the four 1-km² blocks in the core area had four Jackson traps. Traps were placed at the four corners of each square kilometer, 250 m away from the two borders. Each pair of traps in a block row or column was therefore separated by 500 m. Four lines of traps were set from north to south, and within each line, four traps were placed from west to east. In addition to these traps, there were others placed in the buffer zones. B1 had 1 trap/km² located at the center of each block, 12 traps in total. B2 had 8 traps (2.5 traps/km²) evenly spaced around the periphery of 20-km² blocks. The perimeters of the outer buffer zones of the two test plots were separated by a distance of 6 km. Jackson traps were relured with standard trimedlure plugs, each containing 2 ml trimedlure, every 4 wk. Trap placement in the field was carried out using a hand-held geographical positioning system (GPS) Magellan unit with an accuracy of ±30 m (Magellan GPS Systems, Osborne Park, Australia).

Releases of Medfly. Releases of sterile medflies were done using a standard procedure (Wong et al. 1986) consisting of dying the irradiated pupae with a fluorescent dye (DayGlo neon red dye), emerging flies in large paper bags, packing these on a Cessna aircraft, and releasing opened bags over the designated area. This procedure is easily adaptable and minimizes handling damage to the flies. Each of the bags contained ≈15,000 pupae and crumpled paper to provide resting surfaces for emerging flies. As a food source, one square of paper (≈12 by 12 cm) was impregnated with a dried 10% sucrose solution (Nadel et al. 1967). Bags containing pupae were closed by folding the top and stapling. These were placed on wooden racks inside a temperature-controlled room (24 ± 1°C; 65 ± 5% RH) for fly emergence and maintained in darkness during the 3–3.5 d before release.

Release densities per plot per strain were planned to be 4,000 flying adults/ha/wk for the Petapa bisexual strain and 2,000 flying adults/ha/wk for the VIENNA 4/Tol-94 GSS to equalize numbers of males released. Actual released numbers per strain were adjusted up or down in an attempt to balance numbers of males in the field. Quality control data, percent fly emergence from pupae, and adult flight ability in standard tubes provided a means to estimate the numbers of flying insects in the field. The critical ratio of sterile:wild flies observed in field traps was also used, realizing that there would be a variable lag time (estimated to be several weeks) between release dates and dates when

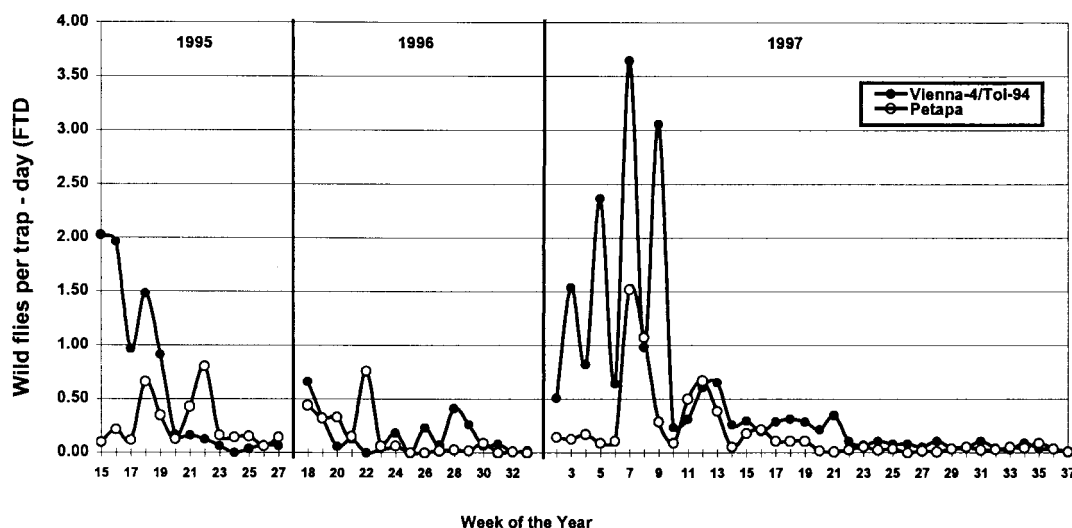


Fig. 2. Estimated numbers of medflies in wild population (flies/trap/d – FTD) in areas where Petapa and VIENNA 4/Tol-94 strains were released (Guatemala, 1995–1997).

the flies would be recaptured. This lag time, in which the wild fly population would also be changing, produced inevitable shortcomings in the attempts to balance male release numbers for each strain. Releases were carried out twice per week (Wednesdays and Sundays) throughout the tests. In the core area (2 km by 2 km), wind-socks were placed in each of the corners of the plot, as well as an additional one in the center of the four blocks.

On each of the mornings of release, 50% of the total bags required for the week were loaded into a temperature-controlled vehicle and transported to the airstrip (10–15 min away). Bags were loaded into the rear of the airplane. Aircraft ferry time from the airstrip to the plot was ≈ 2 min. Fly releases were made by opening the bags with a blunt knife and pushing them through a tube placed at the rear of the aircraft's fuselage. Differences in air pressure ensured that the bag was sucked out of the plane and would land on the target plot. Precision was achieved by flying at optimum speed and altitude (130–150 km/h and 100–150 m, respectively) (Wong et al. 1986) and by paying close attention to the wind socks on the ground for evidence of wind direction and velocity. This procedure was followed twice per week.

Flights were carried out 1 d/wk from south to north (and then the reverse, north to south), with odd numbers of flight lanes first and even numbers second. On the second day of releases, flights were made from west to east. This procedure avoided possible bias in fly distribution. The distance between flight lanes was ≈ 250 m. Releases of sterile insects were carried out from weeks 15–27, 18–33, and 2–37 for years 1995, 1996, and 1997, respectively, with week 1 of each year beginning on 1 January.

Sampling. Adult fly sampling was conducted once per week on the same day in both treatment locations (control area data were usually collected 1 d later).

Samples to determine sterile:wild ratios were taken from the 16 core area traps. Trap inserts were removed from Jackson traps and taken to the laboratory for analysis. Flies were examined under a UV lamp to determine presence or absence of dye, and thus, the strain type. Egg samples for the assessment of induced sterility were taken from coffee berries. Ripe coffee berries were collected and taken to the laboratory where they were held at ≈ 25 – 27°C and 60–70% RH for 2–3 d. Using a stereo-microscope, berries were dissected to find eggs and egg shells. The egg hatch rate from each treatment area was adjusted by subtracting natural egg sterility found in the control area to yield a corrected egg sterility. Radiation-sterilized females do not lay eggs because their ovaries are destroyed by the irradiation process; therefore, all eggs dissected necessarily came from wild females.

Data were analyzed using SAS software, version 6 (SAS Institute 1987). Standard correlations and paired *t*-tests were used where appropriate. In cases where large qualitative differences existed between observed sterility rates, only direct visual comparisons were made.

Results

1995 Evaluation. The development of the wild population of medflies in the two plots over the 3 yr of the tests is presented in Fig. 2. In 1995, the population in Asintal was much higher than that in Palajunuj at the start of releases in week 15, with flies per trap per day values of 2.027 and 0.098, respectively. Releases of sterile insects were carried out from weeks 15–27, with Vienna-4/Tol-94 released in Palajunuj and Petapa flies released in Asintal. Assessment of release rates showed that the actual release densities of flying flies varied slightly throughout the test in both locations (Fig. 3). Sterile:wild ratios increased relatively rapidly

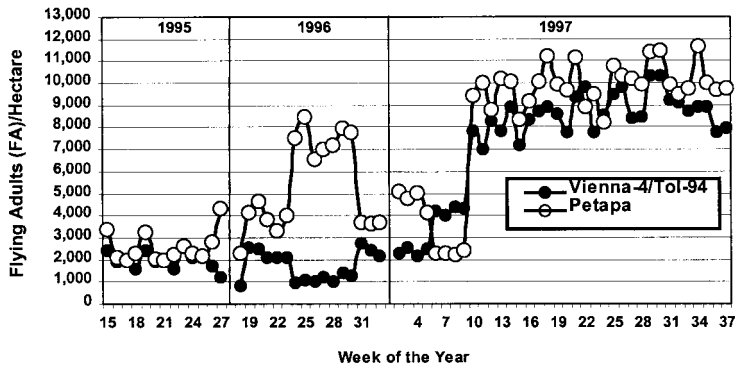


Fig. 3. Estimated released densities of flying adults (FA) medflies/hectare, with releases of Petapa (males and females) and VIENNA 4/Tol-94 (males-only) strains (Guatemala, 1995–1997).

in the VIENNA 4/Tol-94 plot, whereas for the Petapa strain, sterile:wild ratios increased more slowly (Fig. 4). As a result, release rates of the VIENNA 4/Tol-94 strain were decreased during the last weeks of the test (Fig. 3). Despite the reduction in the release rates, sterile:wild ratios for VIENNA 4/Tol-94 from weeks 21–27 were higher than for Petapa by a factor >2.5 (Fig. 4).

During the 1995 releases, the percentage of females in the VIENNA-4/TOL-94 releases gradually increased (Fig. 5) because of genetic instability in the strain. Corrected egg sterility was found to be three to four times more in the VIENNA 4/Tol-94 plot compared with either the control area or the Petapa plot (Fig. 6). Wild fly population levels (Fig. 2) declined from ≈ 2.0 to 0.062 flies per trap per day in week 27.

1996 Evaluation. During 1996, the Petapa strain was released in Palajunoj and VIENNA 4/Tol-94 in Asintal. Initial population levels of wild (fertile) flies were similar in both plots (Fig. 2). Insect releases began in week 18. Release densities (flying adults/hectare) were higher for the Petapa strain than during 1995 (Fig. 3) with a mean (\pm SD) of $5,358 \pm 1,978$. For VIENNA 4/Tol-94, the number released per hectare remained similar to the previous year ($1995 = 1,993 \pm 387$; $1996 = 1,711 \pm 647$). In the Petapa plot, the

resulting sterile:wild ratio was well above that for VIENNA 4/Tol-94 for the last 6 wk of the test (Fig. 4). Also, the percentage of females in the VIENNA 4/Tol-94 releases was significantly higher than in the previous year, especially during the last 7 wk of the evaluation (Fig. 5). This fact, coupled with the higher sterile:wild ratios reached with the Petapa strain, produced similar induced sterility levels for weeks 27–29 in both plots (Fig. 6). However, in weeks 30–33, there was a much higher level of induced sterility in the Petapa plot.

1997 Evaluation. The strains were released in the same plots as in the previous year (Petapa released in Palajunoj; VIENNA 4/Tol-94 released in Asintal). The VIENNA 4/Tol-94 plot had a higher infestation level than the Petapa plot (Fig. 2). Sterile releases began during week 2. The number of flying flies released/hectare (mean \pm SD) was higher than in previous years (Petapa = $8,931 \pm 2,559$ and VIENNA 4/Tol-94 = $7,882 \pm 2,171$), and this led to an increase in sterile:wild ratios. By week 20, sterile:wild ratios had exceeded 100:1 and stayed so for the remainder of the test (Fig. 4). The percentage of females in the releases of VIENNA 4/Tol-94 remained below 1%, because of the introduction of a continuous filter rearing system (Fisher 1996, Fisher and Cáceres 2000). Figure 6

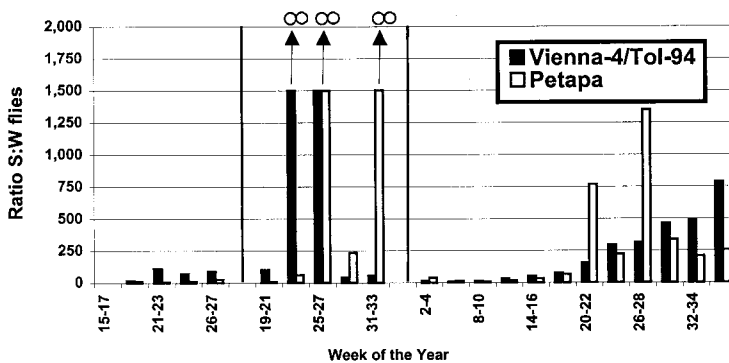


Fig. 4. Ratio of sterile:wild flies (based on FTD capture) during releases of Petapa and VIENNA 4/Tol-94 strains (Guatemala, 1995–1997).

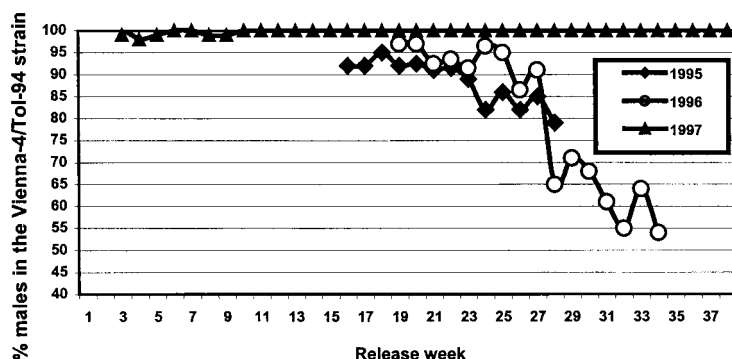


Fig. 5. Percentage of VIENNA 4/Tol-94 strain males in aerial releases (Guatemala, 1995–1997).

shows that the VIENNA 4/Tol-94 plot reached high levels of induced sterility, which were in direct relation to the increase in sterile:wild ratios (Fig. 7). On the basis of the 3-wk interval means during the entire year, sterile:wild fly ratios were highly correlated with corrected egg sterility ($r = 0.82$, $P < 0.01$).

Dispersal. Comparative dispersal and recapture data of Petapa and VIENNA-4/Tol 94 strains in buffer zones, B1 and B2, are presented in Table 1. As can be noted, very similar results were obtained for recaptured male flies of both strains in both buffer zones (paired t -test, $P > 0.05$). Fly recaptures averaged 8.8 and 8.4%, respectively, for VIENNA-4/Tol-94 and Petapa strain in B1, and 0.24 and 0.29%, respectively, in B2. Trapped flies dispersed a minimum of 500 m into B1 and 1,500 m into B2 (see Fig. 1).

Discussion

Large-scale field evaluations of the bisexual (Petapa) and male-only (VIENNA 4/Tol-94) strains were carried out during 3 consecutive yr (1995–1997). The replicates of this evaluation were carried out in time rather than in space, because of logistical problems in dealing with tests of this magnitude and also because of inherent differences among sites.

The release system used for the test has been already superseded by the so-called chilled adult release

system, but the bag system was initially preferred for logistical reasons. To maintain consistency, this release system was used throughout the evaluation. Release of bags in a north-south and east-west pattern weekly and the use of the wind socks produced good results in terms of fly distribution throughout the plot core areas (data not shown). This can be inferred indirectly from the significant levels of fly dispersal into B1 and B2 (Table 1).

With the exception of the second year (1996), in which the bisexual strain achieved higher induced sterility levels during the last weeks of the test, the results suggest that the males-only strain is several times more effective under the test conditions. In the second year, the males-only strain achieved higher levels of induced sterility during 12 of 16 wk, but during the last weeks of the evaluation, the bisexual strain achieved higher induced sterility than expected (based on 1995 and 1997 results). This could be attributed to (1) the higher sterile:wild ratios for the bisexual strain during the final 6 wk and/or (2) the lower percentage of males in the male-only strain, observed during the final 7 wk of the test. It has been documented that there is preferential mating among released flies (Robinson et al. 1986), so it would be expected that a reduction of efficiency in the current releases would occur because of the combined effect of both high presence of females and low male sterile:wild ratios.

During 1997, the male-only strain achieved higher levels of induced sterility, and for both strains sterile:wild ratios were, in general, higher than in previous years. However, the bisexual strain did not induce $>12\%$ sterility, despite sterile:wild ratios exceeding 1,000:1. The difference in sterility levels between males-only and bisexual releases reached a significant level in 1997, with the males-only release achieving 70% induced sterility. However, no significant egg sterility was observed until sterile:wild ratios reached 100:1 or higher (Fig. 7).

An additional improvement over that already observed in favor of the male-only strain would be expected if a reduction of the irradiation dose occurred while maintaining total inherent sterility equal to that for a bisexual strain (Rendón 1996). During the cur-

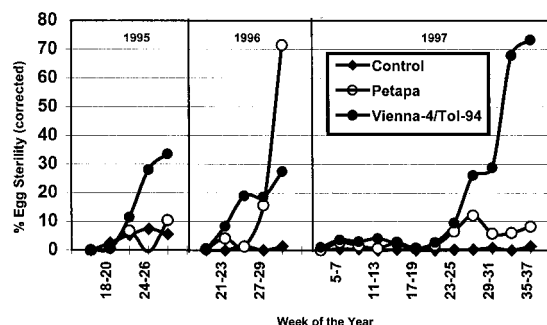


Fig. 6. Comparison of induced egg sterility (corrected) between Petapa and VIENNA 4/Tol-94 strains (Guatemala, 1995–1997).

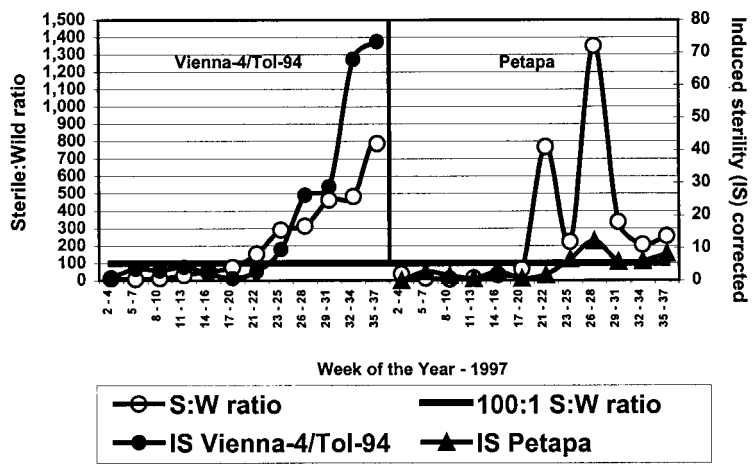


Fig. 7. Comparison of sterile:wild fly ratio in relation to resulting corrected sterility (Guatemala, 1997)

rent tests, the male-only strain was exposed to the same level of radiation as the bisexual strain to compare responses on the basis of an equal level of deleterious radiation. A further general benefit, applicable to both bisex and males-only releases, may come from considering a better fly release and distribution system than the one described above.

Fly dispersal outside the core areas of each treatment plot was virtually the same for both strains—≈9% of released male flies were trapped outside the core areas (Table 1). Therefore, by inference, a high percentage of released flies seem to have been dropped successfully inside the core areas using the described aerial release system. Even though fly dispersal in the core areas was not measured in this study, similar dispersal rates of the sexing and standard strains into their respective buffer areas suggests that, in general, dispersal was also similar in the core areas. Previous studies of dispersal comparing a GSS with a standard strain have found different results. Rendón and coworkers (unpublished data) found no significant difference in dispersal rate using the very same strains in another coffee farm near Guatemala City. In contrast, McInnis and Vargas (1993), in a Hawaii coffee farm, found that males-only flies dispersed signif-

icantly farther compared with males released with females. Apparently, particular strain or environment differences can affect the relative dispersal rates of males-only and bisex strains. It may also be true that net outward dispersal rate, as commonly measured in dispersal studies, is not a good indicator of total male mobility in the field. Random, back-and-forth movements, e.g., while searching for mates, might vary greatly between males-only and bisex strains, yet result in little or no detectable change in net outward dispersal rate.

The results of this 3-yr study strongly support the earlier Hawaii free release field test in which induced egg sterility was monitored (McInnis et al. 1994). Indeed, both studies document a three- to five-fold advantage in the use of males-only medfly releases. Importantly, very similar results were achieved in the current study, despite changing both the strain and the released sex-ratio, unlike in the study of McInnis and coworkers, where the sex-ratio varied, but the strain remained constant. This fact strongly suggests that the three- to five-fold improvement of males-only releases in the current study is not simply caused by a more competitive VIENNA-4/Tol-94 strain. The strength of the current study lies in the fact that it was conducted at a large, program scale level over several years. Sterile flies were aerially released using standard Sterile Insect Technique operational program procedures compared with ground releases of sterile pupae used in the earlier study (McInnis et al. 1994). The clear-cut results of both field studies, strongly in favor of males-only releases, can be compared with several field cage studies conducted in Hawaii, Guatemala, and Chile in the last 10 yr. In general, those latter studies found no significant differences between males-only and bisexual releases when the proportion of females mated by males from the two release strategies was assessed (D.M. and P.R., unpublished data). This would argue that field cages do not appear to adequately simulate open field free-release conditions. By inference, perhaps the major benefit of open field males-only re-

Table 1. Percentages recaptured and minimum distances dispersed of VIENNA 4/Tol-94 and Petapa sterile males into outlying buffer zones (Guatemala, 1997 data only)

Buffer zones	Minimum distance dispersed (m) ^a	Sterile strain	Percentage in buffer zone of total recaptured	Paired <i>t</i> -test (<i>n</i> = 37 weeks) Ho: V-4/ Tol-94 = Petapa
1	500	Vienna 4/Tol-94	8.849	<i>t</i> = 0.795
		Petapa	8.376	(<i>P</i> = 0.447)
2	1500	Vienna 4/Tol-94	0.243	<i>t</i> = -0.858
		Petapa	0.289	(<i>P</i> = 0.413)

^a Minimum distance dispersed by flies to traps in buffer zones 1 or 2, respectively.

leases comes from reduced sterile female "distraction," resulting in heightened mobility in the open field and/or increased male searching behavior for wild females. The observed results provide the necessary evidence to substitute, with confidence, bisexual strain technology with GSS technology for at least medflies, and perhaps for other tephritids.

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